Characterization of the Callipyge Trait in Sheep

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Abstract

In 1983, a sheep producer identified a Dorset ram exhibiting extreme muscular development. When mated with normal ewes, this phenotype was transmitted by the founder ram to part of his offspring and to descendants in later generations, suggesting an inheritable mutation. The name callipyge and symbol CLPG were proposed for the corresponding gene (Cockett et al., 1994). Because the biological action of the callipyge gene is so favorable for meat animals, there has been great interest among animal scientists in determining its biological mode of action. This paper summarizes several research projects that have been initiated to characterize the callipyge trait.

Materials and Methods

Animals

Lambs used in callipyge studies are all descendants of the Dorset founder ram, Solid Gold, with up to 10 generations separating the lambs from the founder. To generate animals for these studies, heterozygous rams that expressed the trait (CLPG/clpg) were mated to normal ewes (clpg/clpg), producing normal and callipyge lambs in 1:1 ratios. Most of the lambs are products of repeated backcrosses, creating animals with up to 97% Rambouillet, Columbia, Hampshire or Suffolk genetics.

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Phenotyping

In most of the studies, including Snowder et al., 1993; Cockett et al., 1994, 1996; Jackson et al., 1996a,b,c and Carpenter et al., 1996, phenotypic classification (normal versus callipyge) was based upon visual evaluation and palpation of the hind legs and of the loin eye muscle for abnormal thickness and size. Accuracy of phenotype determination increases with age of the lamb; therefore, lambs were phenotyped a minimum of three times prior to weaning (90 d).

In the study by Koohmaraie et al., 1995, two groups of lambs were used. Animals within the first group (n=40) were classified as callipyge or normal based on leg scores and *longissimus* area/kg of carcass while the second group (n=20) that was used for analysis of calpastatin levels was classified using visual evaluation. It should be noted that when classification is based on carcass measurements such as leg scores, these measurements cannot be analyzed for differences between normal and callipyge phenotypes.

Genotyping

In the earlier study by Cockett et al. (1994), animals were genotyped with markers GMBT16, CSSM18 and TGLA122. Two additional markers, IDVGA30 and IDVGA39, were added in the later study (Cockett et al., 1996). The genetic marker GMBT16 is a variable number tandem repeat (VNTR) marker and the other four markers are microsatellites.

Results and Discussion

Production Traits

In lambs descending from the founder ram, the muscular hypertrophy phenotype is gradually expressed after approximately three weeks of age. Thus, callipyge lambs do not pose an increased risk of dystocia and there is no evidence of birth weight differences between callipyge and normal lambs (Jackson et al., 1996a).

Expression of the callipyge trait does not appear to influence weaning weight or average daily gain during the post-weaning period (Table 1; Jackson et al., 1996a). How-

TABLE 1. Comparison of Production Traits Between Normal and Callipyge Half-Sibs[1].

Trait	Normal	Callipyge	% Change
Weaning weight (kg)	20.2	20.0	1.0
Average daily gain (kg/d)	0.31	0.29	-6.4
Feed conversion (gain/feed)	0.15	0.17	13.3*
Fleece weight (kg)	6.2	5.5	11.3**
Staple length (cm)	9.2	8.5	-7.6*

[1]Jackson et al.(1996a)
**P < .05*
***P < .01*

TABLE 2. Comparison of Carcass Measurements Between Normal and Callipyge Half-Sibs.

Trait	Normal	Callipyge	% Change
Dressing percentage[1]	51.3	53.6	4.5*
Ribeye area (cm2)[1]	14.8	19.8	33.8*
Leg score[2]	11.0	14.4	30.9**
Total fat (%)[2]	23.6	18.4	-22.0**
Backfat thickness (mm)[1]	6.3	4.5	-29.4*

[1]Koohmaraie et al. (1995)
[2]Jackson et al. (1996a)
**P < .01*
***P < .004*

ever, there is an improvement in feed efficiency and a lower feed intake by callipyge animals when compared to normal lambs (Jackson et al., 1996a), providing an important economic advantage to producers raising callipyge lambs.

There are some negative influences of the callipyge gene on wool traits. In the Jackson et al. (1996a) study, average fleece weight for ewes expressing the callipyge trait was 12.7% lower than the average fleece weight of normal females (Table 1). Also, staple length was 8.7% shorter for callipyge ewes (Table 1). However, it should be noted that the wool production of the callipyge animals was still acceptable by industry standards.

Carcass and Meat Characteristics

Several studies have compared carcass and meat measurements between normal and callipyge animals, including those studies highlighted in Tables 2 thru 4. For most traits, the callipyge carcass is more desirable than the normal carcass. Busboom et al. (1996) recently summarized the results of 11 carcass studies involving 256 normal and 209 callipyge lambs. In these studies, dressing percentage of the callipyge carcass averaged 54.8%, compared to 50.9% for the normal carcass. Rib eye area increased an average 47% and yield of closely trimmed leg, loin, rack and shoulder as a percent of live weight was 11% to 16% greater for the callipyge carcass. Measures of fatness decreased in callipyge lamb carcasses. Back fat thickness at the 12th rib was significantly lower, with fat thickness values of .23 inches in normal lambs compared to .17 inches for callipyge lambs. The subjective measures of shoulder seam fat scores were also significantly lower for callipyge lambs.

Unfortunately, the callipyge *longissimus* muscle is less tender than the corresponding muscle of the normal carcass. This decrease in tenderness has been demonstrated in several ways (Tables 3 and 4). First, in the study by Koohmaraie et al. (1995), calpastatin activity was 82.8% greater in the callipyge *longissimus* compared to normal at time of slaughter and even higher activities at 7 and 21 d post-mortem (86% and 108%, respectively). Also, Warner-Bratzler shear force was higher for the callipyge *longissimus* measured at 1 d, 7 d and 12 d post-mortem and these increases were 44.8%, 112.2% and 144.7%, respectively, of the normal muscle (Koohmaraie et al., 1995). It should be

TABLE 3. Comparison of Meat Characteristics of *Longissimus* Muscles from Normal and Callipyge Half-Sibs[1].

Trait	Normal	Callipyge	% Change
Enzyme activity at death			
μ-calpain	0.9	0.9	0.0
m-calpain	1.2	1.7	45.5*
Calpastatin	3.2	5.8	82.8*
Warner-Bratzler shear force (kg)/1.27 cm core			
1 d post-mortem	7.5	10.9	44.8*
7 d post-mortem	4.7	10.1	112.2*
21 d post-mortem	3.3	8.2	144.7*

[1]Koohmaraie et al. (1995)
**P < .01*

TABLE 4. Comparison of Sensory Characteristics Between Normal and Callipyge Half-Sibs[1].

Trait	Normal	Callipyge	% Difference
Loin (% acceptable)			
Juiceness	90.5	73.3	-17.2**
Tenderness	93.2	65.3	-27.9***
Flavor	93.2	89.3	-3.9
Leg (% acceptable)			
Juiceness	95.9	92.2	-3.7
Tenderness	95.9	92.2	-3.7
Flavor	89.8	86.3	-3.5
Shoulder (% acceptable)			
Juiceness	73.2	78.7	5.5**
Tenderness	97.3	77.3	-20.0***
Flavor	97.3	88.0	-9.3*

[1]Kerth et al. (1995)
**P < .05*
***P < .01*
****P < .0001*

TABLE 5. Comparison of Fibers in the *Longissimus* Muscles from Normal and Callipyge Half-Sibs[1].

Trait	Normal	Callipyge	% Change
Fiber diameter (µm) of longissimus			
Slow, oxidative	28.7	24.3	-15.3*
Fast, oxidative, glycolytic	33.2	38.5	16.0*
Fast, glycolytic	35.5	45.3	27.6*
Fiber content (%) of longissimus			
Slow, oxidative	11.7	7.8	-33.3**
Fast, oxidative, glycolytic	41.8	29.2	-30.1**
Fast, glycolytic	46.5	63.1	35.7**
Fiber diameter (µm) of gluteus medius			
Slow, oxidative	29.9	26.8	-10.4*
Fast, oxidative, glycolytic	36.3	43.4	19.6*
Fast, glycolytic	40.9	48.6	18.8*
Fiber content (%) of gluteus medius			
Slow, oxidative	19.9	12.9	-35.2*
Fast, oxidative, glycolytic	43.1	33.6	-22.4**
Fast, glycolytic	36.9	53.5	45.0**

[1]Carpenter et al. (1996)
**P < .05*
***P < .001*

noted that the shear force of the callipyge *longissimus* after 21 d of aging (8.2 kg) was greater than the shear force of normal *longissimus* after 1 d of aging (7.5 kg).

Interestingly, this increased toughness seems to be limited to the callipyge loin and shoulder, with little or no effect on the leg. In consumer panel evaluations (Kerth et al., 1995), significantly more normal chops from loin and shoulder were rated acceptable for juiciness than callipyge chops (Table 4). Percentage of leg chops rated acceptable for tenderness did not differ between normal and callipyge, but 27.9% more normal chops from the loin and 20.0% more normal chops from the shoulder were rated acceptable for tenderness when compared to the corresponding callipyge chops. Phenotypes did not differ for flavor acceptability in chops from the leg and loin, but 9.3% more normal chops from the shoulder were acceptable than callipyge chops.

There are now several projects underway to study postmortem tenderization methods of the callipyge carcasses. Procedures such as aging (Duckett, unpublished data), electro-stimulation (Carpenter, unpublished data) or calcium chloride infusion (Clare et al., 1995) may be effective in improving the tenderness of callipyge lamb meat.

Carpenter et al. (unpublished data) have conducted a consumer acceptance study with 600 panelists to compare callipyge and normal loin chops. Panelists evaluated their visual likelihood to purchase either of the chops. Seventy-four percent of the panelists were likely or highly likely to purchase callipyge chops while only 27% of the panelists were likely or highly likely to purchase normal chops. Thus, there may be an increased consumer demand for the larger and leaner retail cuts from callipyge lamb carcasses.

Muscle Fiber

The extreme muscular development of callipyge animals appears to be primarily the results of cellular hypertrophy. Using a combined myosin ATPase and NADH tetrazolium reductase stain, Carpenter et al. (1996) found larger average diameters of the fast twitch, oxidative and glycolytic (FOG) and fast twitch, glycolytic (FG) muscle fibers for callipyge muscles, but smaller average diameter for the callipyge slow twitch, oxidative (SO) fibers (Table 5). Fiber content (%) was also examined in that study using callipyge and normal muscles; fiber content was decreased 33.3% and 30.1% for callipyge SO and FOG fibers, respectively, while content increased 35.7% for callipyge FG fibers (Table 5). In contrast, only the percentage of FOG fibers within the *supraspinatus* differed significantly between normal and callipyge muscles, with normal muscle having 40.3% and callipyge having 35.7%. Diameters of the three fiber types did not differ within the *supraspinatus* of the two phenotypes (data not presented).

Economic Value

Expression of the callipyge gene increases total chemical protein by 15% and decreases carcass chemical fat by 25% (Jackson et al., 1996b). Also, the yield of bone-in, untrimmed retail cuts is greater in callipyge carcasses (61.9%) than in normal carcasses (57.5%; Jackson et al., 1996b). These improvements in yield translate into a significant economic advantage for the callipyge carcass. Using a wholesale carcass price of $1.65/lb, Snowder (unpublished data) calculated a $7.94 increase in value for a 130-lb callipyge carcass compared to that of a normal carcass. An overall advantage of $11.95 was calculated for callipyge boxed lamb cuts compared to those obtained from a normal carcass. However, in order for the callipyge gene to be effectively incorporated into current lamb production systems, postmortem tenderization methods must cost less than $7.94 or $11.95 for the whole carcass or boxed lamb, respectively.

Genetics of Callipyge

The callipyge gene has been located on ovine chromosome 18 and positioned by multilocus linkage analysis in the interval between CSSM18 and TGLA122 at 3% recombination from CSSM18 (Cockett et al., 1994). Additional characterization of the callipyge gene has revealed an unusual mode of inheritance (Cockett et al., 1996). To investigate the pattern of inheritance, matings were performed between callipyge ewes (CLPG/clpg) and either (i) normal rams (clpg/clpg) or (ii) callipyge males (CLPG/clpg). Phenotypes (either normal or callipyge) were determined in offspring produced from these matings. All 35 offspring from the first cross (clpg/clpg ♂ x CLPG/clpg ♀) were normal, i.e., none had the extreme muscling typical of the callipyge phenotype. Analysis of microsatellite markers flanking the callipyge locus, how-

TABLE 6. Proposed Phenotypes for Specific Genotypes of the Callipyge Locus[1].

Genotypes	Phenotypes
$clpg^{Pat}/clpg^{Mat}$	Normal
$CLPG^{Pat}/clpg^{Mat}$	Callipyge
$clpg^{Pat}/CLPG^{Mat}$	Normal
$CLPG^{Pat}/CLPG^{Mat}$	Normal

[1]*Cockett et al. (1996)*

ever, demonstrated the expected 1:1 segregation of the corresponding maternal chromosome (i.e. either $clpg^{Mat}$ or $CLPG^{Mat}$) in these offspring. Therefore, these data demonstrate that the reciprocal crosses are not equivalent; that is, CLPG/clpg ♂ x clpg/clpg ♀ matings gave a 1:1 segregation ratio of the callipyge versus normal phenotypes, whereas clpg/clpg ♂ x CLPG/clpg ♀ matings produced only normal offspring.

The second type of cross (matings between heterozygous callipyge rams and ewes) produced 51 offspring. Fifteen (29%) of these were phenotyped as callipyge and 36 (71%) as normal. These numbers differ significantly (χ^2_1 = 56.5, p<0.0001) from the expected 3:1 ratio of callipyge versus normal phenotypes that is expected for the segregation of a dominant mutation in an F2 generation. Analysis of chromosome 18 microsatellite genotypes of these offspring revealed that individuals with genotype $CLPG^{Pat}/clpg^{Mat}$ were callipyge and those with genotypes $clpg^{Pat}/CLPG^{Mat}$ and $clpg^{Pat}/clpg^{Mat}$ were normal, which would be the expected phenotypes based on results from the first cross. In addition, all seven lambs with genotype $CLPG^{Pat}/CLPG^{Mat}$ were normal in appearance.

The callipyge phenotype of $CLPG^{Pat}/clpg^{Mat}$ animals versus the normal phenotype shown by $clpg^{Pat}/CLPG^{Mat}$ individuals reveals the "polar" nature of the CLPG mutation, that is the influence of parental origin on its phenotypic effect. The normal phenotype of $CLPG^{Pat}/CLPG^{Mat}$ animals indicates that the "inactivation" of the callipyge phenotype by the $CLPG^{Mat}$ allele dominates the "activation" of the callipyge phenotype by the $CLPG^{Pat}$ allele. The callipyge locus is therefore characterized by "polar overdominance," where only heterozygous individuals having inherited the CLPG mutation from their sire express the phenotype (Table 6).

To test for the reversible nature of polarity at the callipyge locus, phenotypically normal rams of genotypes $clpg^{Pat}/CLPG^{Mat}$ (n=5) or $CLPG^{Pat}/CLPG^{Mat}$ (n=2) were mated to normal ewes (clpg/clpg). Twenty-three lambs were obtained from the first type of mating ($clpg^{Pat}/CLPG^{Mat}$ ♂ x clpg/clpg ♀). Thirteen (56.5%) of these lambs were classified as callipyge and 10 (43.5%) as normal, pointing towards reactivation of the CLPG mutation after passage through the male germline. Matings between the normal-appearing $CLPG^{Pat}/CLPG^{Mat}$ rams and normal clpg/clpg ewes produced 33 lambs of which 30 (91%) were callipyge and three were normal, suggesting reactivation of the CLPG alleles in homozygous rams as well. The three normal offspring were obtained from one of the two rams, suggesting that the reversibility of the callipyge polarity is not absolute in all rams.

Conclusions

The callipyge mutation offers sheep producers a unique opportunity to increase lean meat production and decrease overall fat content within the lamb carcass, with extra advantages of increased feed efficiency and decreased feed intake. However, these advantages are somewhat counterbalanced by the decreased tenderness of the callipyge *longissimus* muscle. Before the wide-spread production of callipyge animals is promoted, economical methods for tenderizing the callipyge loin should be determined. Several methods are currently being investigated and will likely prove successful.

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